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Influence of Wear and Overwear on Surface Properties of Etafilcon A Contact Lenses and Adhesion of *Pseudomonas aeruginosa*

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PURPOSE. To determine changes in physicochemical surface properties of contact lenses (CLs) during daily wear and effects of lens wear on adhesion of a *Pseudomonas aeruginosa* strain from a patient with CL-related keratitis.

METHODS. Ten new CL wearers used ionic, etafilcon A lenses with 58% water on both eyes for approximately 10 hours each day during 10 and 50 days. All lenses were treated daily with an appropriate lens care solution. After the CLs were worn for 10 days (first pair of lenses) and 50 days (second pair, representing overwear), hydrophobicity by water contact angles, surface roughness by atomic force microscope, elemental surface composition by x-ray photoelectron spectroscopy (XPS), and adsorbed proteins by SDS-PAGE were determined on one lens. The lens from the contralateral eye was placed in a parallel plate flow chamber for bacterial adhesion after each time interval.

RESULTS. Water contact angles on lenses changed from 45° on unused lenses to 61° ± 25° after 10 days of wear and changed significantly ($P < 0.05$) to 27° ± 14° after 50 days of wear. Surface roughness increased significantly ($P < 0.05$) from 4 ± 2 nm (unused) to 10 ± 7 nm after 50 days of wear. These changes were accompanied by adsorption of proteinaceous material, as evidenced by XPS and SDS-PAGE, demonstrating adsorption of lysozyme, tear lipocalin, and a 30-kDa protein. Initial bacterial adhesion to worn CLs was lower than to unworn CLs. Furthermore, detachment of adhering bacteria from worn lenses was easier than from unworn lenses. The changes observed in the physicochemical surface properties of the lenses after the CLs were worn for 50 days were accompanied by reports of discomfort by 6 of the 10 new CL wearers. Multiple regression analysis revealed that the most predictive variables for an effect on initial deposition after 10 days of wear were hydrophobicity, roughness, the presence of nitrogen-rich material, including the presence of a 30-kDa protein, and the presence of oxygen-rich material—that is, the type of oxygen adsorbed (O=C or O—C). After 50 days of wear, roughness and the presence of tear lipocalin were most predictive.

CONCLUSIONS. This study demonstrates that the physicochemical surface properties changed after wear and overwear,

whereas overwear of the lenses decreased initial adhesion of *P. aeruginosa* #3 under the present experimental conditions. (*Invest Ophthalmol Vis Sci.* 2002;43:3646–3653)

The adhesion of *Pseudomonas aeruginosa* to soft contact lenses (CLs) is an important initial step in the pathogenesis of microbial keratitis.¹ Microbial keratitis is a serious complication of CL wear and may result in permanent visual damage to the cornea. For daily wear, regular cleansing and disinfection procedures must be applied to minimize the risk of biofilm growth. However, organisms able to survive in ophthalmic solutions^{2,3} may adhere to lens surfaces and thus be transported to the eye. Extended-wear CLs, although handled less frequently with associated risks of contamination, are also less frequently subject to protective hygienic procedures.⁴ The possibility of daily or extended wear bears the risk of overwear—that is, wearing lenses longer than the prescribed term, which is a potential risk factor for ocular health.⁵

CLs are bathed in tear fluid during wear, containing an abundance of different proteins that adsorb momentarily after insertion of a lens in the eye⁴ with a potential impact on bacterial adhesion. In addition, lens care solutions leave adsorbed components after cleansing. By consequence, adhesion of infectious bacteria never occurs on a bare CL surface, but always on a so-called conditioning film of adsorbed macromolecular components.^{6,7} Adsorption of conditioning film components changes the physicochemical properties of the CL surfaces, such as hydrophobicity, electrostatic charge, and surface roughness. Conditioning films on CLs can be expected to be composed of lipids, salts, proteins, and different components of lens care solutions. Recently, the adsorption of O—C-rich components to CL surfaces has been demonstrated to enhance bacterial deposition.⁸ However, it is unknown how the CL surface is conditioned after various cycles of tear film formation and exposure to a lens care solution. Although in separate studies changes in elemental surface composition of lenses have been determined by x-ray photoelectron spectroscopy (XPS) after 10 minutes of wear,⁹ changes in surface roughness by atomic force microscopy (AFM) after 5 minutes to 48 hours of wear,¹⁰ and bacterial interactions with lenses worn for 6 hours,¹¹ these different types of surface characterization measurements after clinically relevant periods of wear and overwear have never been combined in one study.

Therefore, the purpose of this study was to determine changes in physicochemical surface properties after wear and overwear of Etafilcon A lenses and effects on adhesion of *P. aeruginosa* #3 to worn lenses in a single study. This allowed us to determine the effects of the changes in hydrophobicity, surface roughness, chemical composition, and protein adsorption during lens wear on bacterial adhesion through multiple regression analysis.

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MATERIALS AND METHODS

Bacterial Strain, Growth Condition, and Harvesting

P. aeruginosa #3, a clinical isolate from a patient with CL-related keratitis was obtained by courtesy of Donald G. Ahearn (Georgia State University, Atlanta, GA). A frozen stock was precultured in 10 mL tryptone soya broth (TSB; Oxoid, Basingstoke, UK) for 24 hours at 37°C in ambient air. A preculture was used to inoculate a second culture (200 mL) for 18 hours at 37°C in ambient air in 250-mL Erlenmeyer flasks to yield midexponential-phase cells. *P. aeruginosa* was harvested by centrifugation for 5 minutes at 9600g, washed twice with ultrapure water, and resuspended in 10 mL ultrapure water. For adhesion experiments, *P. aeruginosa* #3 was suspended to a density of 3×10^8 cells/mL in 0.9% saline, supplemented with 2% (wt/vol) TSB to stimulate their adhesion but preventing their growth in suspension.¹²

Volunteers and Contact Lenses

Ten volunteers (four men and six women), with no previous experience in wearing CLs, wore two pairs of etafilcon A CL (Surevue, made of a cross-linked copolymer of 2-hydroxyethyl methacrylate with 2% methacrylic acid, ionic, 58% water, Vistakon; Johnson & Johnson, Jacksonville, FL). The volunteers' ages ranged between 20 and 55 years, and they were given CLs with a power between -1.00 D and -5.00 D. One pair was used on both eyes for approximately 10 hours each day during 10 days, and a second pair was used during 50 days, representing overwear (prescribed maximum period of wear is 30 days). During wear, a commercially available and appropriate lens care solution (Opti-Free; Alcon, Fort Worth, TX) was used.

Volunteers had a good corneal health, as established by inspection of the cornea, conjunctiva, and eyelid. Compatibility with wearing a CL was determined with the Schirmer break-up time test, and the fit of the CLs was determined through keratometry by a qualified optometrist. In addition, all volunteers were educated about regular cleansing and hygiene. After 10 or 50 days of wear, CLs from both eyes of each period of wear were collected into lens cases after which the cases were filled with CL care solution and directly transported to the laboratory. After removal from the lens cases, lenses were dipped five times in 0.9% saline, and excess saline was removed by gently tapping the lens on a paper tissue. One lens of each pair of lenses per volunteer and per period of wear was cut in four parts that were used for measurement of hydrophobicity, elemental surface composition, or surface roughness or for analysis of adsorbed proteins by SDS-PAGE. The other lens of each pair—that is, from the contralateral eye—was placed in a parallel plate flow chamber for bacterial adhesion.

Three pairs of unused lenses were used as a control. After removal of these lenses from their packages, the lenses were also dipped five times in 0.9% saline, as described. As described for the worn lenses, one lens of each new pair was cut into pieces for the measurement of hydrophobicity, elemental surface composition, and surface roughness, whereas the other lens was examined for bacterial adhesion.

After the lenses were collected, volunteers were asked whether they had experienced a difference in general wearing comfort after 10 or 50 days.

All procedures were approved by the Medical Ethics Committee of the University Hospital, Groningen, The Netherlands. The protocol included informed consent from the volunteers and adhered to the tenets of the Declaration of Helsinki.

Contact Angle Measurements

Hydrophobicity of the CL was determined by the measurement of water contact angles. Contact angles were measured immediately after the lenses were cut, to prevent dehydration. One CL quarter was put on a microscope slide, and measurements of advancing type water

contact angles on the convex side were performed by the sessile drop technique in air at 25°C.¹³

X-ray Photoelectron Spectroscopy

Contact lens surface chemistry was determined by XPS (S-probe; Surface Science Instruments, Mountain View, CA) before and after wear. Wet CL quarters were placed in the XPS machine with their convex sides up and dried in the prevacuum chamber. X-rays (10 kV, 22 mA), at a spot size of $250 \times 1000 \mu\text{m}$, were produced with an aluminum anode. Scans of the overall spectrum in the binding energy range of 1 to 1200 eV were made at a low resolution (pass energy, 150 eV), and then peaks over a 20-eV binding energy range were recorded at high resolution (pass energy, 50 eV) for C_{1s}, O_{1s}, N_{1s}. The area under each peak was used to calculate peak intensities, yielding elemental surface concentrations for carbon, oxygen and nitrogen, after correction with sensitivity factors provided by the manufacturer. In addition, the oxygen peak was divided into two components, representing oxygen involved in O=C bonds (set at 531.3 eV), and oxygen bonded otherwise, including O—C bonds (set at 532.6 eV).¹⁴

Atomic Force Microscopy

For AFM, hydrated lens quarters were fixed on a microscope slide with double-sided sticky tape after cutting. The AFM (Nanoscope IIIa Dimension 3100; Digital Instruments, Santa Barbara, CA) was operated in the contact mode with an Si₃N₄ cantilever tip of 0.06 N/m spring constant. Height images were recorded in three dimensions at three randomly selected sites on one CL quarter, from which the mean roughness (R_a) was calculated.

Polyacrylamide Gel Electrophoresis

Adsorbed tear film components were extracted from the CL surfaces by incubating lens quarters in 100 μL electrophoresis buffer (1 mM EDTA, 10 mM Tris-HCl [pH 8.0], 2.5% SDS, and 5% β -mercaptoethanol). After boiling for 15 minutes and centrifugation at 10,000g for 10 minutes, the supernatant was applied to 10% to 15% gradient gels and run on the electrophoresis unit (Phast System; Pharmacia, Uppsala, Sweden).^{10,15} The protein bands were visualized by an automated silver-staining procedure, using the kit reagents (Phast Gel Silver Kit; Pharmacia) according to the manufacturer's instruction, and image enhancement. In addition to the analysis of adsorbed tear film components, human tears collected from volunteers and low-molecular-mass protein standards, including phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), and α -lactalbumin (14.4 kDa), were used. Identification of other proteins, including lysozyme and tear lipocalin, was made on the basis of molecular weight comparisons.^{10,15}

Flow Chamber and Deposition Protocol and Enumeration

A parallel plate flow chamber (dimensions [length \times width \times height] = $17.5 \times 1.7 \times 0.075 \text{ cm}$) and image analysis system were used to study bacterial adhesion to whole CLs.¹⁶ Contact lenses were fixed with the convex side up on a poly (methyl methacrylate) bottom plate of the flow chamber. To avoid edge effects, a shallow, circular section was removed from the bottom plates to fit a CL. The flow chamber was positioned on the stage of a phase-contrast microscope (BH-2; Olympus, Birkeroed, Denmark) equipped with a 40 \times ultra-long-working-distance objective (ULWD-CD Plan 40 PL; Olympus), and images were taken randomly over the surfaces of CLs. The number of bacteria adhering to the CL were observed over a surface area of a 0.017-mm² field of view, with a charge-coupled device camera (CCD-MX; High Technology, Eindhoven, The Netherlands) mounted on the microscope, and enumerated.

Before each experiment, all tubes and the flow chamber were filled with 0.9% saline, while care was taken to remove all air bubbles from

TABLE 1. Water Contact Angles, Surface Roughness Values, and Percentage Elemental Surface Compositions and Oxygen Ratios of a Soft, Hydrogel Contact Lens after 10 and 50 Days of Wear by 10 Different Volunteers

	θ_w	R_A	%C	%O	%N	$\frac{O=C}{O-C}$
Unused	45	4	72	27	0	2.9
Ten days of wear						
1	51	10	70	23	7	1.3
2	93	3	70	20	10	1.9
3	43	4	67	24	6	0.7
4	110	4	68	23	8	1.6
5	61	3	66	22	10	2.1
6	35	*	70	20	9	1.5
7	59	4	72	25	3	1.5
8	66	*	72	22	6	1.2
9	27	7	72	24	4	0.4
10	68	3	75	16	6	2.2
Mean \pm SD	61 \pm 25	5 \pm 2	70 \pm 3	22 \pm 3	7 \pm 2	1.4 \pm 0.6
Fifty days of wear						
1	18	22	64	19	9	1.7
2	15	5	78	15	7	1.5
3	*	*	*	*	*	*
4	26	3	64	21	12	2.0
5	31	20	70	22	8	1.4
6	17	10	67	19	12	2.8
7	18	2	76	18	6	2.0
8	55	8	72	19	9	1.7
9	43	15	63	23	10	1.9
10	18	7	71	22	8	1.8
Mean \pm SD	27 \pm 14	10 \pm 7	69 \pm 5	20 \pm 2	9 \pm 2	1.9 \pm 0.4

Data include means \pm SD over the group of volunteers. Results for unused lenses were obtained in triplicate, yielding a standard error of 10° for the water contact angle, 10% for elemental surface concentration, and 2 nm for surface roughness.

*Not measured.

the system. Flasks containing bacterial suspension and saline were connected to the flow chamber. At a relatively low shear rate of 10/sec (flow rate 0.016 mL/sec) the fluids circulated through the flow chamber under the influence of hydrostatic pressure. First, the flow was switched to 0.9% saline for 5 minutes. Thereafter, the bacterial suspension was circulated through the system for 2 hours, and the number of bacteria adhering to the CL was determined. After this deposition period, flow was switched to saline to remove nonadhering bacteria from the tubes and the flow chamber. Subsequently, the number of adhering bacteria before and after passing an air-liquid interface (i.e., an air bubble) over the bacteria were compared, to mimic the natural shear-off action of blinking of the eyelid. During wear, the wiping motion of the lid causes thinning of the tear film between blinks,⁹ which leads to the formation of a three-phase boundary between air, an adhering bacterium, and the lens surface. This three-phase boundary is associated with a surface tension detachment force, as theoretically analyzed by Leenaars and O'Brien,¹⁷ and experimentally examined for its ability to detach adhering bacteria from surfaces by Gómez Suárez et al.¹⁸

After enumeration of the number of adhering bacteria in a field of view, data were transformed to number of bacteria per unit area. For each experiment, an initial deposition rate per unit time and area, j_0 (approximately representing the first 30 minutes of an experiment) and number of bacteria adhering after 2 hours per unit area (n_{2h}) were calculated, as well as the percentage of bacteria removed after the passage of an air-bubble.

Statistical Analyses

To analyze differences between variables measured after lenses were worn for 10 and 50 days, paired *t*-tests were performed on computer (SPSS for Windows; SPSS Inc., Chicago, IL), with a significance level of 0.05. Backward multiple linear regression was performed to identify the surface properties most predictive of bacterial adhesion. The initial deposition rate was used as a dependent variable, and the water

contact angle, surface roughness, percentage of surface composition (%C, %O, %N), and the ratio between the two types of oxygen distinguished $\frac{O=C}{O-C}$ were taken as independent variables. The absence or presence of small- (<14 kDa) and high- (30 kDa) molecular-weight proteins and of tear lipocalin were entered as independent variables after transformation into 0 or 1. Variables were excluded when equal for all volunteers or if they correlated significantly ($P < 0.05$) with other variables, as determined with the Pearson correlation test.

RESULTS

Surface Characterization

The changes in physicochemical surface properties of the hydrogel CL during wear are compiled in Table 1. Before use, the lenses possessed an intermediate hydrophobicity, and the water contact angles amounted to $45^\circ \pm 10^\circ$. During the first 10 days of wear, water contact angles decreased in 3 of 10 CL users, but mostly an increase was observed, to $61^\circ \pm 25^\circ$ on average. After overwear, however, CL surfaces became significantly ($P < 0.05$) more hydrophilic, with an average water contact angle of $27^\circ \pm 14^\circ$. These changes in hydrophobicity were accompanied by changes in lens surface chemistry. Surfaces of unused CLs contained carbon and oxygen as their major constituents, whereas after the lenses were worn, adsorption of conditioning film components was indicated by increased amounts of nitrogen. After the CLs were worn for 10 and 50 days, the percentage surface nitrogen (%N) increased from 0% to an average of 7% and 9% ($P < 0.05$), respectively. However, in some users decreases in %N were seen when comparing lenses worn for 50 days with those worn for 10 days. Simultaneously, the percentage surface oxygen (%O) decreased significantly ($P < 0.05$) after the lenses had been worn

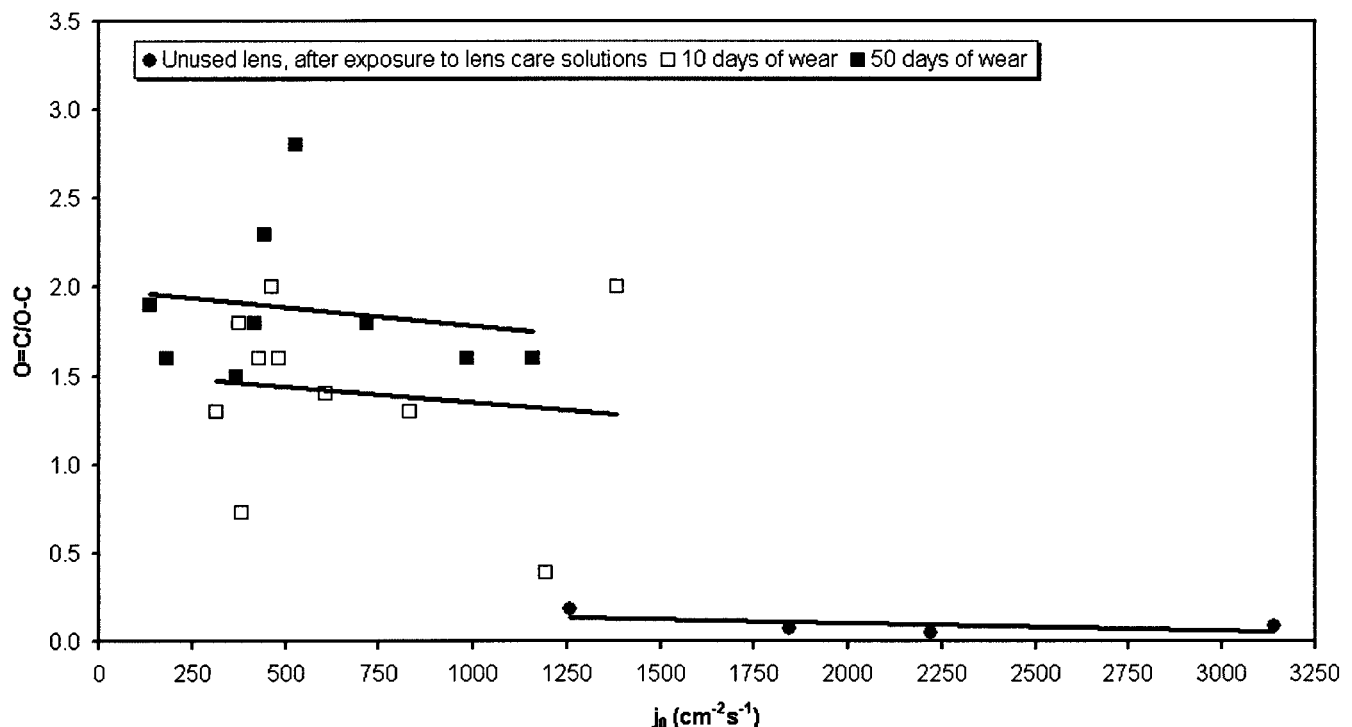


FIGURE 1. The ratio of adsorbed oxygen-rich components as a function of initial adhesion rates of *P. aeruginosa* #3 to worn CLs and to lenses after exposure to four lens care solutions.⁸ Data points refer to different volunteers or solutions.

for 10 days, compared with the surface of an unused CL, and continued to decrease significantly ($P < 0.05$) after 50 days. The percentage surface carbon (%C) decreased slightly, but this decrease was not significant. Decomposition of the oxygen peak in $O=C$ or bound otherwise, including $O-C$ bonds, showed that different ratios of oxygen-rich components were left adsorbed after CLs were worn for 10 days compared with 50 days of wear. The ratio between the two types of oxygen bonds ($O=C/O-C$) varied between 0.4 and 2.2 after 10 days of wear in different volunteers, and became more similar, between 1.5 and 2.8, in all individuals after 50 days (Table 1, Fig. 1). In Figure 1, previously published data for unused etafilcon A lenses exposed to lens care solutions are included for comparison,⁸ showing that the ratio of $O=C/O-C$ of unused lenses after exposure to lens care solutions varied between 0.05 and 0.18.

The mean surface roughness of an unused CL is 4 nm and did not change significantly over the first 10 days of wear

(Table 1). However, after CLs were worn for 50 days, the mean surface roughness increased to 10 ± 7 nm.

Table 2 summarizes the absence or presence of specific proteins, as demonstrated by SDS-PAGE, in tears of each volunteer and on their lenses after 10 days and 50 days of wear. Lysozyme is present on lenses collected from all volunteers, regardless of the duration of the period of lens wear. Albumin and lactoferrin or secretory component, although present in tears of all volunteers, was never found adsorbed on a lens. Other proteins found on lenses of some but not all volunteers and after different periods of wear were tear lipocalin and a low- (<14 kDa) and high- (30 kDa) molecular-weight protein, likely to be the dimer of lysozyme.¹⁹

Bacterial Adhesion

Bacterial adhesion data are summarized in Figure 2 and demonstrate a high individual variation among lenses worn by

TABLE 2. Proteins Identified Using SDS-PAGE in Tears of 10 Volunteers and from Contact Lenses after Wear for 10 or 50 Days

	1			2			3			4			5			6			7			8			9			10		
	T	10	50	T	10	50	T	10	50	T	10	50	T	10	50	T	10	50	T	10	50	T	10	50	T	10	50	T	10	50
<14 kDa	+	-	-	+	+	-	+	-	+	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
LZ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30 kDa	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	+	+	-	+	+	-	-	-
AL	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-
LF/SC	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-

T, tears; 10 and 50, 10 and 50 days off wear respectively; <14 kDa and 30 kDa, presence of low- and high-molecular-mass protein bands; LZ, lysozyme; TL, tear lipocalin; AL, albumin; LF/SC, lactoferrin or secretory component. Lenses after 10 and 50 days of use are indicated by a volunteer number.

*Not measured.

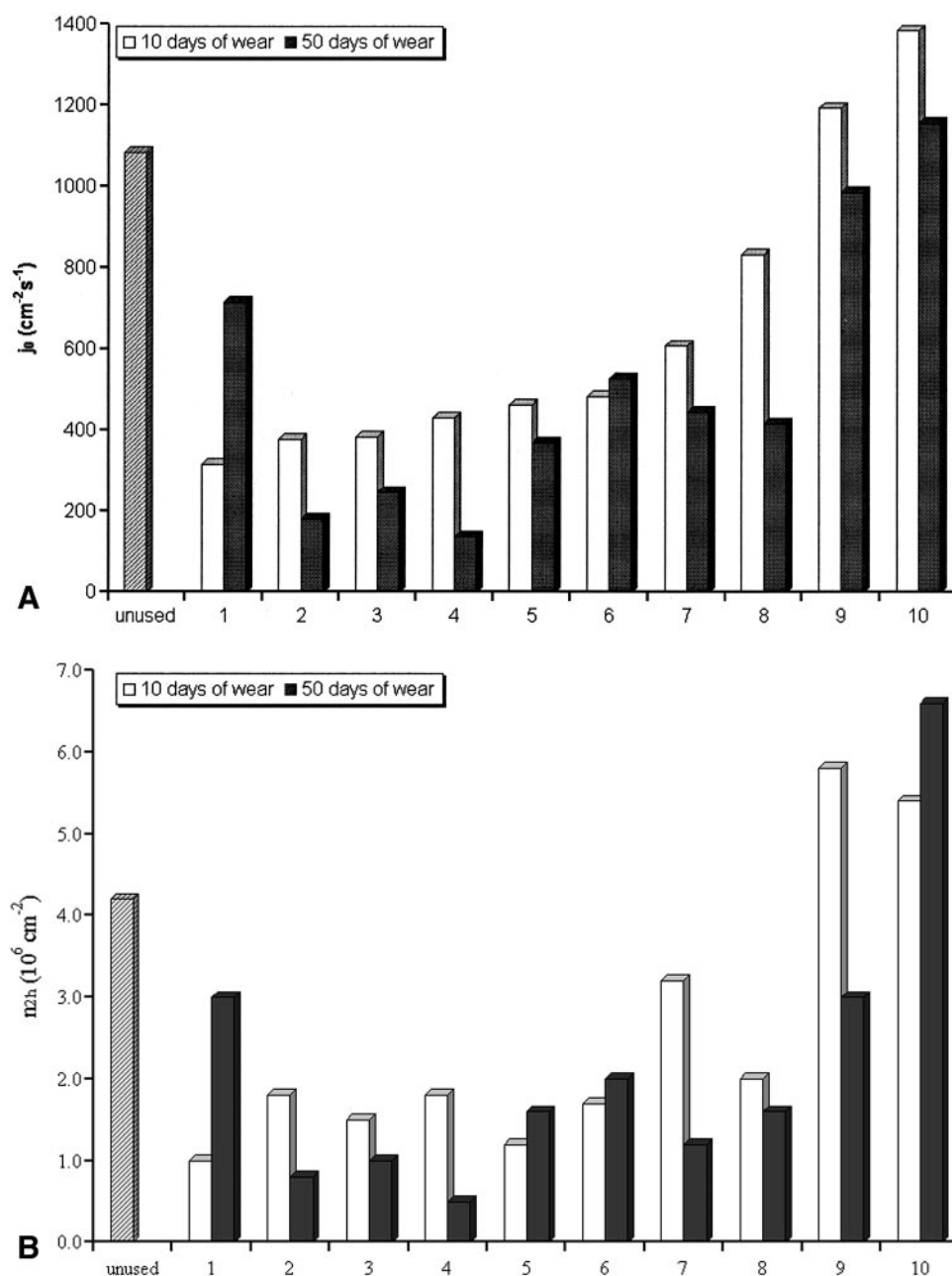


FIGURE 2. Adhesion in a parallel plate flow chamber of *P. aeruginosa* #3 to soft CLs after 10 and 50 days of wear in 10 volunteers. (A) Initial (representing approximately the first 30 minutes of an experiment) deposition rate. (B) Number of bacteria adhering after 2 hours. (C) Percentage detachment stimulated by the passage of an air bubble through the flow chamber. Results in unused lenses were obtained in triplicate from three different lenses, yielding an SD of 30%.

different volunteers. In 8 of the 10 CL users, initial deposition rates (Fig. 2A) of *P. aeruginosa* #3 decreased after the lenses were worn for 10 days ($P < 0.05$), when compared with an unused lens, whereas initial deposition rates were even lower after 50 days of wear ($P < 0.05$). The number of bacteria adhering after 2 hours was lower after 10 days of wear in 8 of the 10 users, whereas in 5 of these 8, still lower numbers were seen after the CLs were worn for 50 days (Fig. 2B). Concurrent with this decrease in adhesion of *P. aeruginosa* #3 to the 50-day lens surface, the percentage detachment stimulated by the passage of an air bubble through the flow chamber increased (Fig. 2C), confirming the decreased affinity of the strain for the CL surface. In addition, Figure 1 demonstrates that after the CLs were worn for 10 and 50 days, a decreasing trend was seen between initial deposition rates and the ratio between the two types of oxygen bonds ($\text{O}=\text{C}/\text{O}-\text{C}$), similar

to the trend observed for unused lenses, although correlations are weak.

Wearing Comfort

None of the new users reported any discomfort from wearing the CLs over the initial 10 days. After 50 days of use, however, 6 of the 10 new CL wearers reported discomfort, most notably a grazed feeling, redness of the eye (conjunctiva), and general irritation.

Multiple Regression Analysis

The Pearson correlation test demonstrated for the variables obtained after 10 days of wear a high correlation between %C and the presence of a protein band at less than 14 kDa with the other variables, and hence they were excluded from backward

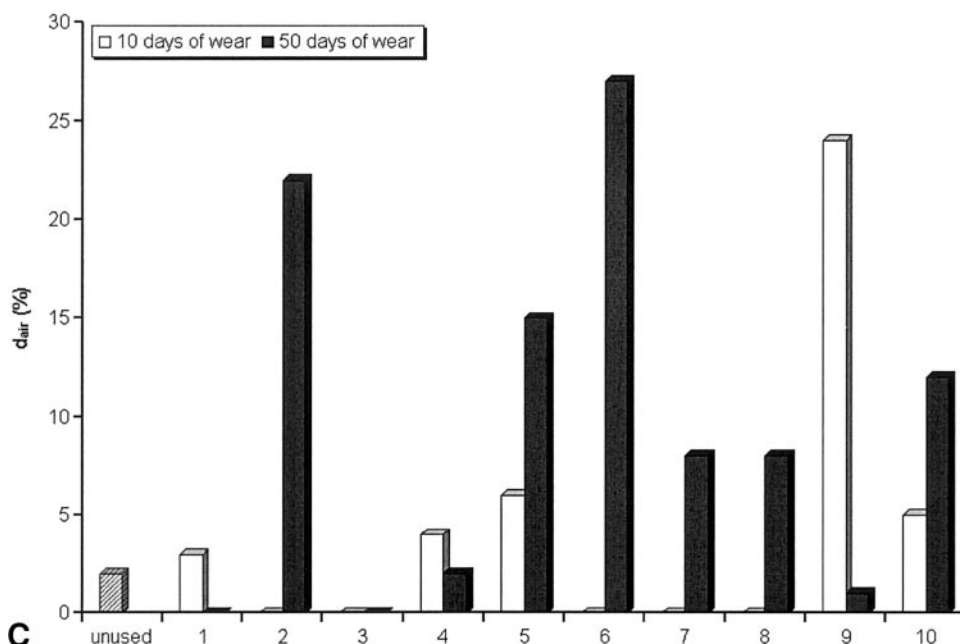


FIGURE 2. (Continued)

multiple regression analysis. Similarly, for the data obtained after 50 days of wear, the %C and the presence of protein bands less than 14 kDa and 30 kDa were excluded from regression analysis. Lysozyme was excluded from multiple regression analysis because it was present on lenses of all volunteers.

Table 3 shows that after 10 days of wear, hydrophobicity, surface roughness, %O, %N, $\text{O}=\text{C}/\text{O}-\text{C}$ ratio, and a 30-kDa protein band were jointly predictive of adhesion of *P. aeruginosa* with a slightly stronger impact of %O and %N than of the other variables. The presence of tear lipocalin was not a predictive variable for initial adhesion. The number of predictive variables was greatly reduced, however, after the lenses were worn for 50 days, and surface roughness and the presence of

tear lipocalin became equally dominant factors that jointly accounted for 49% of the total variance (R^2) in initial deposition rate. Consequently, the intercept values and unstandardized coefficients resulting from these multiple regression analyses yielded the following equations, describing initial deposition of *P. aeruginosa* on lenses worn for 10 and 50 days, respectively.

$$j_0 = 5677 + 10 \times \theta_w - 108 \times R_A - 160 \times \%O - 191 \times \%N - 417 \times \text{O}=\text{C}/\text{O}-\text{C} + 787 \times 30 \text{ kDa} \quad (1)$$

and

$$j_0 = 666 + 30 \times R_A - 547 \times \text{TL} \quad (2)$$

where TL is tear lipocalin.

DISCUSSION

Adsorption of conditioning film components to surfaces in the human body tends to change the hydrophobicity of the surface. Surfaces in the human oral cavity tend to obtain water contact angles between 50° and 70°, because of the adsorption of salivary proteins and dentifrice components.²⁰ In this study, progressive exposure of CL surfaces with an intermediate hydrophobicity to the ocular environment yielded hydrophilic surfaces due to adsorption of tear film and lens care solution components. Considering the multitude of physicochemical and molecular changes occurring on the CL surface during wear, it becomes unlikely that a single surface characteristic—for instance, hydrophobicity—could on its own explain bacterial adhesion. Indeed, as a virtue of this study, which comprised a number of different experimental techniques, our multiple regression analyses indicate that bacterial adhesion to worn lenses is a multifactorial process. Whereas hydrophobicity had a weak influence on bacterial deposition, regardless of the duration of use, the increase in surface roughness from 4 nm for an unused lens to 10 nm after the CLs were worn for 50 days makes roughness an influential surface characteristic (Ta-

TABLE 3. Standardized Coefficients and Probabilities after Backward Multiple Regression Analysis

Input	10 days wear		50 days wear	
	β	P	β	P
θ_w	0.37	0.03	2	2
R_A	-0.66	0.02	0.63	0.1
%C	1	1	1	1
%O	-1.13	0.01	2	2
%N	-1.19	0.01	2	2
$\text{O}=\text{C}/\text{O}-\text{C}$	-0.63	0.02	2	2
<14 kDa	1	1	1	1
TL	2	2	-0.70	0.08
30 kDa	0.99	0.02	1	1
R^2	100%		49%	

Analysis takes the initial bacterial deposition rate as the dependent variable and water contact angle (θ_w), surface roughness (R_A),

C, %O, %N, $\text{O}=\text{C}/\text{O}-\text{C}$ ratio and the presence of different proteins (<14 kDa, tear lipocalin (TL), and 30-kDa band) as independent variables. Unstandardized coefficients (B) are presented in Eqs. (1) and (2).

¹These input variables were found to have a correlation with other variables, as determined with the Pearson correlation test. ²These variables do not play a significant role in determining the initial deposition of *P. aeruginosa*.

ble 3). As another feature, the presence of nitrogen, to be interpreted as the presence of adsorbed proteins, lost its protective influence after the CLs were worn for 50 days compared with wear for only 10 days. The bacterium used in this study is, of course, only one representative of organisms that are potentially pathogenic to the eye, in that it was isolated from a patient with a CL-related infection. However, it was chosen for this study for its extreme hydrophobicity,¹⁶ causing it to adhere tenaciously to surfaces, regardless of their hydrophilicity of the surface.²¹ This is the probable reason that the increased hydrophilicity observed during progressive wear of the lenses shows a relatively weak relationship with bacterial adhesion in multiple regression analysis. It cannot be ruled out that less hydrophobic and pathogenic organisms would show different results. Because cell surface hydrophobicity is generally considered to be a virulent factor in infection,²² the use of such an extremely hydrophobic strain was preferred.

Although hydrophilicity of the CL surfaces has been associated with increased comfort,²³ 60% of the volunteers considered the lenses more uncomfortable after wearing them for 50 days, when the largest changes toward hydrophilicity were observed (Table 1). Changes in surface roughness have also been suggested to affect the comfort of wearing CLs,²⁴ but it is hard to imagine that an increase in roughness by 6 nm, as observed here after 50 days, would cause discomfort. However, Michaud and Giasson⁵ showed in their clinical study that overwear increases discomfort, which may lead to deleterious effects on ocular health. At this point, it is noted that although 50 days exceeds the recommended maximum 30 days of wear, more than half of all visitors to our Ophthalmologic Clinic, by their own choice, prefer to wear the lenses longer than 30 days. XPS indicated the presence of nitrogen-rich, proteinaceous material on the lenses. XPS, however, is less sensitive than, for instance, water contact angles, and its depth of detection reached up to 15 nm in a materials' surface under the conditions applied in this study. Hence, the nitrogen detected is not only due to adsorbed conditioning film components but also to conditioning film components absorbed in the CL material. McArthur et al.⁹ showed that increased amounts of nitrogen could be detected on CL surfaces after only 10 minutes of wear, indicating the speed at which the formation of conditioning film proceeds. However, our studies clearly demonstrate that conditioning film formation is not completed after 10 minutes of wear,⁹ because even differences in physicochemical surface properties of the lenses are observed after 10 and 50 days, due to continued adsorption of tear film components and components from the lens care solutions.

The present results indicate that lenses do not become more prone to bacterial adhesion after the maximal wear time has been exceeded, and consequently the chance of developing microbial keratitis does not increase with a longer wearing period. Possibly this is because natural defensive tear film components, probably lysozyme, are abundantly present on and in the lenses, as demonstrated in this study by SDS-PAGE. Although albumin and lactoferrin were not detected on the lens surfaces by SDS-PAGE, their presence has not been ruled out, because these proteins may well have been too tightly adsorbed to the lenses for detection in SDS-PAGE. Willcox et al.¹¹ and Boles et al.²⁵ also showed that bacterial adhesion was less after wear of etafilcon A CLs than with unused lenses, although the conclusion by Boles et al. was based on only four volunteers. Our results show that large differences may exist between different volunteers, as in the study by Willcox et al.

As a difference between our study and these previous ones, we measured adhesion under in situ conditions, whereas others in fact measured bacterial retention by counting bacteria on

the worn lenses after removal of weakly adhering bacteria by washing and dipping. Consequently, most of the data in the literature are more comparable with the results presented in Figure 2C, after the passage of an air bubble through the flow chamber.

To summarize, this study shows that a large number of physicochemical surface properties of etafilcon A CLs change during wear (10 days) and overwear (50 days), with an impact on bacterial adhesion. Important from the point of view of infection, these changes in surface properties during wear and even overwear do not increase the adhesion of *P. aeruginosa*, one of the most frequently isolated pathogens that cause CL-related keratitis.

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